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A Randomized Controlled Trial of Atorvastatin in Patients With Bronchiectasis Infected With *Pseudomonas Aeruginosa*

A Proof of Concept Study

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BACKGROUND: There are no randomized controlled trials of statin therapy in patients with severe bronchiectasis who are chronically infected with *Pseudomonas aeruginosa*.

METHODS: Thirty-two patients chronically infected with *P aeruginosa* were recruited in this double-blind cross-over randomized controlled trial. Sixteen patients were recruited in each arm, were given atorvastatin 80 mg or placebo for 3 months followed by a washout period for 6 weeks, and then crossed over and administered the alternative therapy for 3 months.

RESULTS: Twenty-seven patients completed the study. Atorvastatin did not significantly improve the primary end point of cough as measured by the Leicester Cough Questionnaire (mean difference, 1.92; 95% CI for difference, –0.57-4.41; $P = .12$). However, atorvastatin treatment resulted in an improved St. Georges Respiratory Questionnaire (–5.62 points; $P = .016$) and reduced serum levels of CXCL8 ($P = .04$), tumor necrosis factor ($P = .01$), and intercellular adhesion molecule 1 ($P = .04$). There was a trend toward improvement in serum C-reactive protein and serum neutrophil counts ($P = .07$ and $P = .06$, respectively). We demonstrated in vitro that atorvastatin 10 μM reduced formyl-methionyl-leucyl phenylalanine-induced upregulation of CD11b expression and changes in calcium flux, reflecting an ability to decrease neutrophil activation.

CONCLUSIONS: We demonstrated that atorvastatin reduced systemic inflammation and improved quality of life in patients with bronchiectasis who were infected with *P aeruginosa*. These effects may be due to an ability of atorvastatin to modulate neutrophil activation.

TRIAL REGISTRY: ClinicalTrials.gov; No.: NCT01299194; URL: www.clinicaltrials.gov

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KEY WORDS: infection; inflammation; microbiology

ABBREVIATIONS: CRP = C-reactive protein; fMLF = formyl-methionyl-leucyl phenylalanine; LCQ = Leicester Cough Questionnaire; SGRQ = St. George's Respiratory Questionnaire

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Bronchiectasis is characterized by permanently damaged airways and persistent excessive neutrophilic airway inflammation; yet despite this, there is ongoing bacterial colonization. The kinetics of the establishment of infection and its relationship to the subsequent inflammatory response is poorly understood.[1] The severity of the inflammatory response depends on the interplay between proinflammatory mediators, which are upregulated, and anti-inflammatory mediators and inhibitors, which are released to limit the extent and duration of inflammation.[2] We hypothesize that there is a failure of resolution of inflammation in bronchiectasis and propose a role for anti-inflammatory therapy in bronchiectasis.

Statin drugs have been used for more than 2 decades for primary prevention of cardiovascular disease. However, over the past few years, studies in both animal and human models have established that statin drugs have pleiotropic effects, which include modulation of the innate and adaptive immune system and anti-inflammatory properties.[3,4] The mechanisms by which statin drugs modulate inflammation remain incompletely defined.

We recently demonstrated that 6 months of treatment with atorvastatin, 80 mg once daily, reduced cough, enhanced sputum neutrophil apoptosis, and reduced serum CXCL8 (also called interleukin [IL] 8) when used in the stable state in patients with moderately severe bronchiectasis who were not chronically infected with *Pseudomonas aeruginosa*.[5] These encouraging results prompted a trial of atorvastatin treatment in a group of patients with more severe bronchiectasis who were chronically infected with *P aeruginosa*.

We hypothesized that prolonged statin treatment would improve patients' symptoms as a consequence of the anti-inflammatory properties of the drug. The aims of this study were the following:

1. To assess whether atorvastatin 80 mg once daily for 3 months could reduce cough severity and inflammation in patients with bronchiectasis who were chronically infected with *P aeruginosa*.
2. To assess the mechanisms (in vitro studies) by which statin drugs may modulate neutrophilic inflammation in patients with bronchiectasis.

Methods

Study Population

We recruited bronchiectasis patients aged 18 to 79 years who were receiving treatment at the Royal Infirmary of Edinburgh, Scotland. Inclusion criteria were chronic cough and sputum production when the patients were clinically stable, two or more exacerbations in the preceding year, chronic infection with *P aeruginosa* (defined as two or more isolates of *P aeruginosa* when the patients were clinically stable [ie, no exacerbations requiring antibiotics] in the 12 months before the study), and bronchiectasis confirmed on chest CT imaging by a radiologist. For diagnosis on CT imaging, bronchial dilatation had to be present (bronchus to arterial ratio $>$ 1). The Bronchiectasis Severity Index was calculated in all patients.[6]

We excluded current smokers or former smokers who had stopped smoking $<$ 1 year previously and those with a $>$ 15 pack-year history or those with predominant emphysema on CT imaging; patients with cystic fibrosis, active allergic bronchopulmonary aspergillosis, active tuberculosis, or poorly controlled asthma; those who were pregnant or breastfeeding; patients with a known allergy to statin drugs; those currently receiving statin drugs or who had used them within the previous year; patients with active malignant disease or chronic liver disease; those receiving long-term oral macrolide therapy (because of the known interaction with statin drugs); and patients with active inflammatory disease (arthritis, bowel disease) requiring disease-modifying agents.

West of Scotland Research Ethics Committee (14/WS/1080) approved the study. All patients gave written informed consent.

Randomization and Masking

We randomly allocated patients to receive either high-dose atorvastatin (80 mg) or placebo (lactose), given orally once a day for 3 months. Following this, patients had a 6-week washout period (half-life of atorvastatin is 14-20 hours). Patients were then crossed over and commenced the opposite treatment. Although the placebo was not matched to atorvastatin in appearance, the study medicines were dispensed by and returned to the pharmacy; hence, allocation concealment was always maintained for the study investigators. Random allocation sequence in block randomizations of four was done.

Outcomes

The primary outcome measure was reduction in perceived cough at 3 months compared with baseline, measured by the Leicester Cough Questionnaire (LCQ) score. Secondary outcomes included FEV_1, FVC; incremental shuttle-walk test; qualitative and quantitative sputum bacteriological results; frequency of exacerbations; health-related quality of life (St. George's Respiratory Questionnaire [SGRQ]); assessment of sputum neutrophil numbers and apoptosis; neutrophil activation in the airway measured by sputum myeloperoxidase, free elastase activity, and CXCL8; systemic inflammation measured by WBC count, C-reactive protein (CRP), and erythrocyte sedimentation rate; other markers of systemic inflammation, including concentrations of IL-1β, IL-6, CXCL8, IL-10, IL-12p70, tumor necrosis factor (TNF), and intercellular adhesion molecule 1 (ICAM-1); and safety of treatment.

Procedures

Clinical studies: We conducted assessments at baseline, 3 months, $4^1/_2$ months, and $7^1/_2$ months (duration of treatment was 3 months, as this was a cross-over study). If study subjects had an exacerbation, they were examined and assessed at the beginning and end of the exacerbation.

We assessed cough with the LCQ. This questionnaire is a 19-item self-completed quality of life measure of chronic cough, with scores from 3 to 21 (a lower score indicates more severe cough). The minimum clinically important difference in the LCQ score is 1.3 units.[7-9]

We measured prebronchodilator FEV_1, FVC, and FEV_1/FVC by spirometry, followed by an incremental shuttle-walk test—an externally paced 10-m field-walking test.[10] Health-related quality of life was assessed with the SGRQ, which is a 50-item self-administered test with a total score ranging from 0 to 100 (a higher score indicates poorer health-related quality of life). The minimum clinical important difference in SGRQ score is 4 units.[11]

We induced sputum with hypertonic (3%) saline for 10 min. Sputum was induced in all patients to ensure that appropriate samples were obtained and that there was no patient to patient variability (ie, patients not producing sputum at the time of study visit or patients producing inadequate samples). Samples were used for bacteriological analysis and neutrophil assessments.[12] We used 1 mL of the sputum sample for qualitative and quantitative microbiological analyses. The rest of the sample was used to assess total cell numbers and analysis of the activity of myeloperoxidase, free neutrophil elastase, and CXCL8.

We took 30 mL of venous blood to obtain a full blood count; erythrocyte sedimentation rate; levels of CRP, urea, electrolytes, and creatine kinase; and liver function tests. Serum was stored for measurement of proinflammatory and anti-inflammatory cytokines and chemoattractants by cytometric bead array (BD Biosciences) and enzyme-linked immunoassays per the manufacturers protocols (CXCL8 and ICAM 1 [R&D Systems]).

We assessed patients for the presence or absence of side effects at all study visits. If the activity of alanine aminotransferase was greater than five times the normal value or if concentrations of creatine kinase were greater than three times the upper limit of normal, we stopped the assigned study treatment. We recorded all side effects on a patient diary card. We defined exacerbations according to the British Thoracic Society guidelines (increased cough, increased sputum volume or purulence, or both, and feeling systemically unwell), treated them according to baseline sputum bacteriological findings, and administered 14 days of oral or IV antibiotic treatment per British Thoracic Society guidelines.[13] If their study assessment days coincided with an ongoing exacerbation, patients were reviewed and assessed when stable within 2 weeks of completing the antibiotic therapy. Statin drugs were not stopped during an exacerbation.

In vitro studies: Isolation of neutrophils: Granulocytes were isolated by dextran sedimentation and discontinuous Percoll gradient, as described, from blood taken from healthy volunteers.[14]

Neutrophil activation: As there was a reduction in serum ICAM-1 (see Results section), we investigated the role of statin drugs in CD11b expression, which functionally regulates neutrophil adhesion. We also assessed expression of CD62L, which is key for leukocyte rolling prior to migration. After neutrophils were isolated, they were treated with atorvastatin at varying concentrations. Fluorescein isothiocyanate-labeled CD11b antibodies and phycoerythrin-labeled CD62L antibodies were added and samples were analyzed by flow cytometry.[15]

Intracellular calcium flux: As Ca^{2+} ions serve as important second messengers in signal transduction in neutrophils, we investigated the role of statin drugs in regulating neutrophil calcium flux. Neutrophils were loaded with fura-2/AM (Invitrogen). Intracellular calcium flux was quantified in response to formyl-methionyl-leucyl phenylalanine (fMLF), with or without a 30-min pretreatment with atorvastatin at varying concentrations.[16] Please see e-Appendix 1 for full details of in vitro studies methods.

Statistical Analysis

Based on previous studies, we know the mean (SEM) preadministration/postadministration change in placebo is −0.23 (1.1 units) and that a difference of 1.3 units is a clinically relevant difference.[7-9] We used a two-sided paired test with a 5% level of significance, 80% power, and a mean of difference of 1.3. The sample size was 26 subjects. To account for an approximate 20% dropout rate, we recruited 32 patients.

We analyzed the study with a modified intention-to-treat model. For demographic and clinical variables, we presented data as mean (SD) for continuous variables and number (%) for categorical variables, unless otherwise stated. To examine continuous variables, we calculated the change during the atorvastatin period (either baseline-3 months or $4^1/_2$-$7^1/_2$ months) and compared this to the change during the placebo period (either $4^1/_2$-$7^1/_2$ months or baseline-3 months] by a paired *t* test. We did not take the washout period into account. To compare the proportion of patients with either clinical improvement (measured by the LCQ) or quality of life gains (measured by the SGRQ), we used a McNemar test. We compared categorical data between groups with the χ^2 test. Statistical significance was taken to be $P < .05$. We analyzed all data with SAS, version 9.4 (SAS Institute) and GraphPad Prism, version 6.0f (GraphPad Software, Inc.).

Results

Thirty-two patients were randomized to receive treatment: 16 received atorvastatin 80 mg and 16 received placebo for 3 months (Fig 1). Baseline demographics are shown in Table 1. Data are presented as mean (SEM).

Primary Outcome

There was no evidence of a difference in the mean LCQ change in patients treated with atorvastatin compared with those treated with placebo (mean difference, 1.92; 95% CI for difference, −0.57-4.41; $P = .125$). During the active period, 12 of 27 participants showed a clinically relevant improvement in the LCQ (≥ 1.3 units) compared with five of 27 subjects in the placebo group; 10 improved only while receiving atorvastatin, three improved only while receiving placebo, and two improved during both periods. However, using a McNemar test, there was no evidence of a difference in the distribution of the discordant results at the 5% level ($P = .092$).

Secondary Outcomes

Twenty-seven patients completed the study and were included in the modified intention-to-treat model in the secondary outcome analysis.

Quality of life: There was a significant difference in the change in SGRQ total score for atorvastatin compared with placebo (mean difference, −5.62; 95% CI for difference, −10.13 to −1.13; $P = .016$). There was an

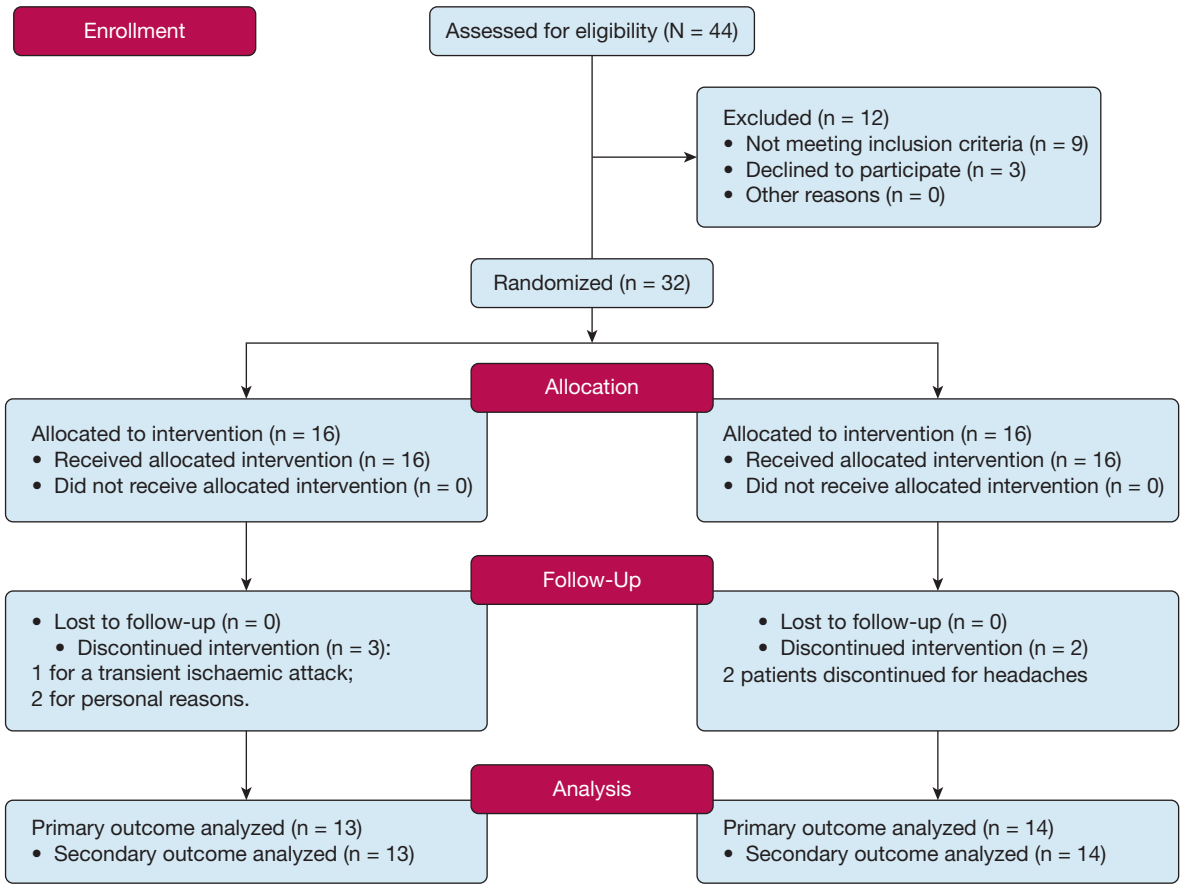

Figure 1 – *Consort diagram of recruitment.*

improvement in the activity domain of the SGRQ in patients receiving atorvastatin compared with those receiving placebo (mean difference, –5.7; 95% CI for difference, –10.3 to –1.1; $P = .02$). Data for other domains are not shown.

Serum inflammatory markers: There was evidence of a significant difference in the change in serum CXCL8 ($P = .04$), TNF ($P = .01$), and ICAM1 ($P = .04$) at the end of treatment in atorvastatin-treated subjects compared with those given placebo (Fig 2, Table 2). There was no significant change in serum IL-1β ($P = .3$), IL-6 ($P = .3$), IL-10 ($P = .6$), or IL-12p70 ($P = .1$).

The mean change in CRP and serum neutrophil counts between the two treatments showed a trend toward reduction in the atorvastatin group but failed to reach statistical significance ($P = .07$ and $P = .06$, respectively). There was no evidence of a significant difference in the change in erythrocyte sedimentation rate in atorvastatin compared with placebo ($P = .7$) at the end of treatment (Table 2).

Sputum microbiology: In the atorvastatin group, 94% had a positive sputum sample with bacteria at the beginning of treatment and 77% had a positive result at the end of treatment; in the placebo group, the numbers were 69% and 75%, respectively. Fewer patients had a positive sputum sample in the atorvastatin group compared with those in the placebo group at the end of treatment (comparison within groups, $P = .03$).

In patients who remained infected in the atorvastatin group, the mean (SEM) quantitative bacterial count at the beginning of treatment was 7.6 (0.16) log units and at the end of treatment it was 7.2 (0.26) log units; in the placebo group, the numbers were 7.3 (0.27) log units and 7.3 (0.24) log units, respectively. There was no significant decrease in bacterial load between the groups at the end of treatment ($P = .1$).

Sputum inflammatory markers: There was no change in the number of apoptotic neutrophils ($P = .9$), eosinophils, basophils, or monocytes (Table 2). There

TABLE 1] Baseline Demographics

Variable	Atorvastatin-Placebo n = 16	Placebo-Atorvastatin n = 16
Age, y, mean (SEM)	62.3 (2.4)	67.8 (2.5)
Sex, female, %	66	66
BMI, kg/m^2, mean (SEM)	25.2 (1.1)	25.9 (0.7)
BSI (SEM)	11.4 (0.9)	10.9 (0.9)
Smoking status, No. (%)		
Nonsmoker	13 (81)	11 (69)
Ex-smoker	3 (19)	5 (31)
Cause of bronchiectasis, No. (%)		
Idiopathic	11 (69)	12 (75)
Postinfection	3 (19)	2 (12)
Rheumatoid arthritis	0 (0)	1 (6)
Ulcerative colitis	2 (12)	1 (6)
Spirometric findings, mean (SEM)		
FEV_1, L	1.7 (0.2)	1.8 (0.2)
FEV_1, % predicted	54.6% (9.4)	58.4% (6.5)
FVC, L	2.5 (0.2)	2.8 (0.2)
FVC, % predicted	62.1% (10.2)	72.9% (7.8)
Sputum microbiological findings, No. (%)		
Pseudomonas aeruginosa	10 (64)	9 (57)
Haemophilus influenzae	2 (12)	0 (0)
Gram-negative bacteria	2 (12)	2 (12)
Streptococcus pneumoniae	1 (6)	0 (0)
Mixed normal flora	1 (6)	5 (31)
COPD, No. (%)	0 (0)	1 (6)
Asthma, No. (%)	4 (25)	5 (31)
ABPA (inactive), No. (%)	2 (12)	2 (12)
Previous malignancy, No. (%)	4 (25)	5 (31)
Hypertension, No. (%)	2 (12)	5 (31)
ICS, No. (%)	11 (69)	11 (69)
Long-term oral steroids, No. (%)	0 (0)	0 (0)
Long-term antibiotic for chest, No. (%)	5 (31) (3 receiving inhaled tobramycin, 2 receiving inhaled gentamicin)	3 (19) (2 receiving inhaled gentamicin, 1 receiving inhaled tobramycin)

ABPA = allergic bronchopulmonary aspergillosis; BSI = bronchiectasis severity index; ICS = inhaled corticosteroids.
[a]*P aeruginosa* was not isolated in some patients at the start of the study, but they met the criteria of being chronically infected with *P aeruginosa* (defined as two or more isolates of *P aeruginosa* while clinically stable in the 12 months before the study) and thus could be coinfected with other microorganisms.

was no statistical improvement in sputum CXCL8, myeloperoxidase, or free neutrophil elastase levels (Table 2).

Lung physiology and exercise tolerance: There was no improvement with atorvastatin in FEV_1, FVC, or FEV_1/FVC, or in the incremental shuttle-walk test (Table 2).

Cholesterol levels: There was a statistically significant improvement in cholesterol levels (mean difference, −1.6 mmol/L; $P < .0001$) in the atorvastatin group.

Exacerbations requiring antibiotics: Eleven of 27 patients (41%) had exacerbations during both periods. During the placebo phase seven of 27 subjects (26%) had exacerbations, and during the active phase, nine of 27

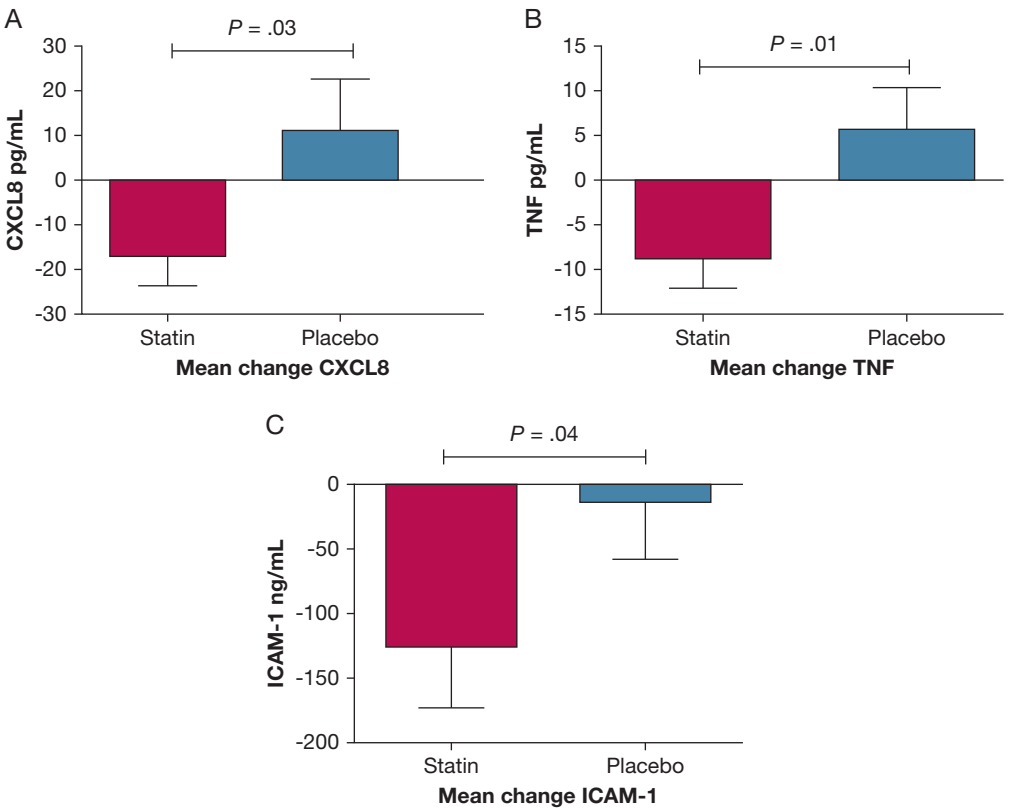

Figure 2 – *A-C, Serum inflammatory markers. Serum CXCL8 (A), serum TNF (B), and serum ICAM-1 (C), showing mean change and SEM. CXCL8 = interleukin 8; ICAM-1 = intercellular adhesion molecule 1; TNF = tumor necrosis factor.*

subjects (33%) had exacerbations. There was no improvement in exacerbations with atorvastatin treatment.

Adverse Events

There was no significant difference in the creatinine kinase or alanine aminotransferase levels while subjects received statin drugs (Table 2). Three patients dropped out when they were receiving the active treatment and two patients dropped out while they were in the placebo group. Of the three patients who dropped out of the active group, one patient had to withdraw, as he had a transient ischemic attack (unrelated to the study drug), and two subjects withdrew for personal reasons. Both patients in the placebo group dropped out because of headaches. No other adverse events were recorded during the study.

In Vitro Studies

Neutrophil activation: As there was a reduction in serum ICAM-1, we investigated the role of statin drugs in CD11b expression, which functionally regulates neutrophil adhesion, and found that atorvastatin 10 μM significantly reduced fMLF-induced peripheral blood neutrophil activation ($P = .03$). This effect was comparable to a known potent and competitive inhibitor of formyl peptide receptor-1 (FPR1)—cyclosporin H ($P = .03$) (Fig 3A,C). There was, however, no effect of atorvastatin 10 μM on CD62L shedding (Fig 3B).

fMLF induced Ca^{2+} flux: fMLF (10 nM) induced a significant rise in calcium flux. This fMLF-induced increase in cytoplasmic Ca^{2+} was significantly reduced by preincubation (30 min) with atorvastatin (area under the curve, 0.7 for atorvastatin 10 μM (SE, 0.04; $P < .0001$) in a concentration-dependent manner (Fig 4A,B).

Discussion

This proof of concept study showed that although administration of atorvastatin for 3 months to patients with bronchiectasis infected with *P aeruginosa* did not reduce cough, it did improve quality of life, as assessed by the SGRQ. In addition, this treatment reduced systemic inflammation, evidenced by a statistically significant reduction in serum CXCL8, TNF, and ICAM-1, at the end of 3 months of statin treatment. A trend toward a reduction of serum neutrophil count and CRP levels was also observed, but this failed to reach statistical significance. Furthermore, atorvastatin

TABLE 2] Results Comparing the Change on Active Treatment vs Placebo

Parameters Measured	Mean	95% CI		*P* Value
Induced sputum				
Apoptotic neutrophils, %	0.111	−11.328	11.550	.98
Eosinophils, %	0.519	−0.161	1.198	.13
Monocytes, %	0.074	−0.913	1.061	.88
Neutrophils, %	22.000	−29.652	73.652	.39
Pulmonary physiological measures				
FEV_1, L	0.007	−0.117	0.131	.90
FVC, L	−0.058	−0.106	0.223	.47
FEV_1/FVC ratio	−0.013	−0.056	0.031	.55
Blood markers				
CXCL8, pg/mL	−27.96	−54.95	−0.96	.04
TNF, pg/mL	−14.24	−25.63	−2.85	.01
ICAM-1, ng/mL	−126.67	−249.61	−3.72	.04
ALT, IU/L	2.769	−3.133	8.672	.34
Urea, mmol/L	−0.412	−1.163	0.340	.27
Creatinine, μmol/L	−3.385	−9.216	2.446	.24
Creatinine kinase, U/L	0.125	−53.450	53.700	1.0
Cholesterol, mmol/L	−1.642	−2.168	−1.117	< .0001
CRP, mg/L	−14.104	−29.561	1.353	.07
ESR, mm/h	−1.272	−10.889	8.344	.76
WBC, $\times 10^9$/L	−0.485	−1.338	0.368	.25
Neutrophils, $\times 10^9$/L	−0.602	−1.246	0.041	.06
Eosinophils, $\times 10^9$/L	0.060	−0.042	0.162	.23
Basophils, $\times 10^9$/L	−0.007	−0.068	0.053	.80
Lymphocytes, $\times 10^9$/L	0.065	−0.184	0.314	.6
Monocytes, $\times 10^9$/L	−0.013	−0.138	0.111	.83
Sputum markers				
CXCL8, pg/mL	−7,255.300	−21,249.100	6,738.400	.3
Myeloperoxidase, ng/mL	−16,709.400	−47,540.700	14,121.800	.27
Neutrophil elastase, ng/mL	12,144.600	−28,571.500	52,860.700	.54
Exercise capacity				
Incremental shuttle-walk test, m	5.2	−45.6	56.1	.62

ALT = alanine aminotransferase; CRP = C-reactive protein; CXCL8 = interleukin 8; ICAM-1= intercellular adhesion molecule 1; TNF = tumor necrosis factor.

treatment resulted in a statistically significant reduction in the number of patients with pulmonary bacterial colonization compared with placebo, although no significant change in mean bacterial load was observed. This is perhaps secondary to the anti-inflammatory effect alone of statin drugs. There was no improvement in spirometric findings, exercise capacity, or sputum inflammatory markers.

Our current study did not show statistical evidence of achieving its primary end point: cough reduction. We have previously shown that atorvastatin reduced cough severity as measured by the LCQ when administered for 6 months to 60 patients with bronchiectasis who were infected with microorganisms other than *P aeruginosa*.[5] However, in this current study, treatment was for only 3 months, the size of the study group was smaller, and a group of patients with more severe bronchiectasis was studied. The proof of concept study period was shorter to check its safety in this group with more severe bronchiectasis.

Although the LCQ did not change, there was an improvement in the quality of life, as measured by the

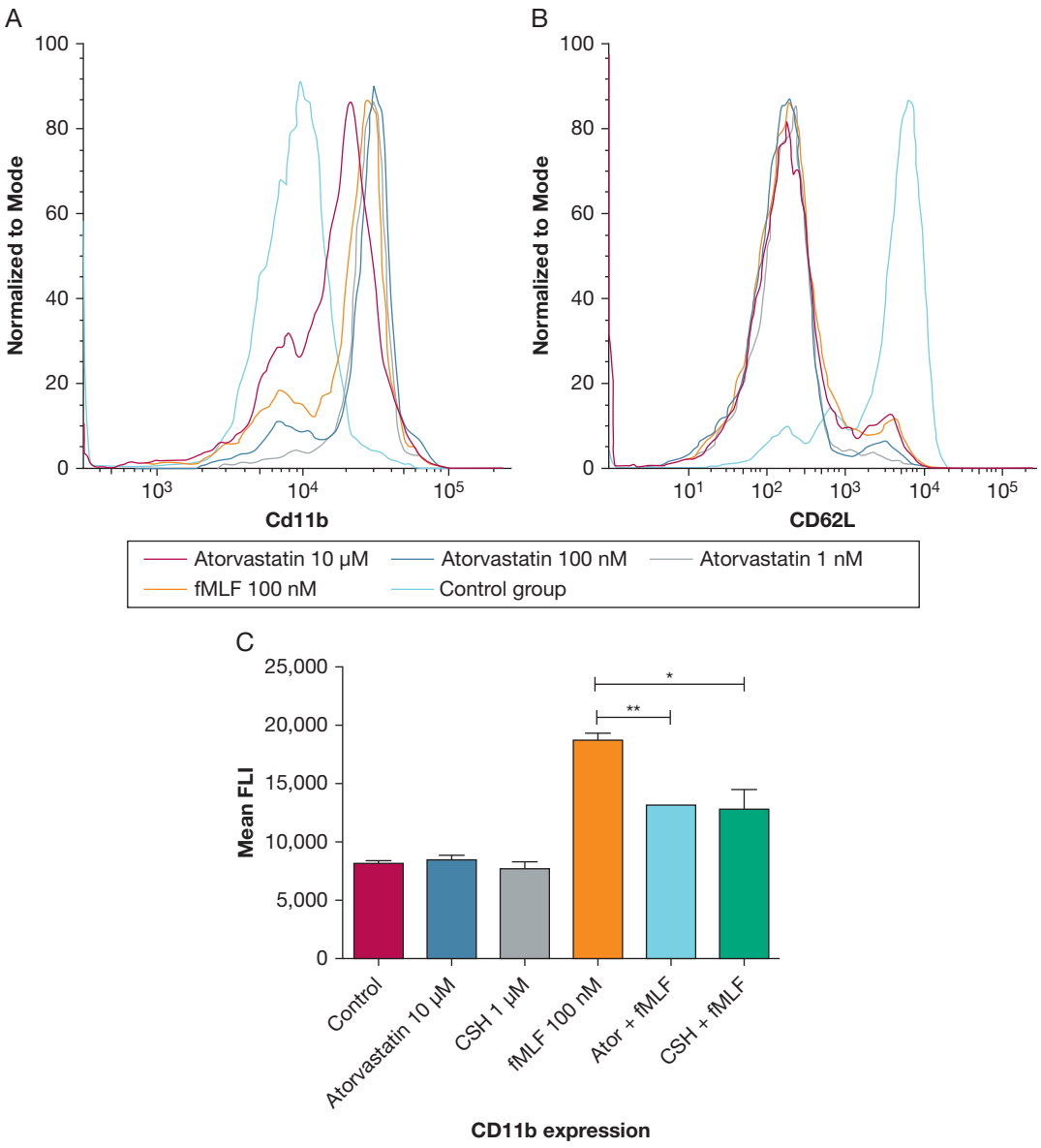

Figure 3 – *A-C, fMLF-stimulated serum neutrophils increased CD11b expression, and this was significantly reduced when neutrophils were pretreated with 10 μM of atorvastatin (**P = .02). This was comparable to the positive control in the experiment—CSH, a known formyl peptide receptor 1 (FPR1) antagonist; *P = .03. B, There was no effect on fMLF-induced CD62L shedding. C, Cumulative data from N = 5 experiments showing that atorvastatin and CSH were able to reduce fMLF induced CD11b expression. Ator = atorvastatin; CSH = cyclosporin H; FLI = fluorescence index; fMLF = formyl-methionyl-leucyl phenylalanine.*

SGRQ. There was an improvement in the activity domain in the SGRQ score. The LCQ focuses on cough-related quality of life, whereas the SGRQ is a more generic respiratory quality of life score that assesses symptoms, activity, and impact. The mechanism that enabled statin drugs to improve the activity domain is unknown.

It has been well established that statin drugs can reduce the expression and function of molecules on the leukocyte's surface.[17] Statin-sensitive cellular functions include adhesion, chemotaxis, and release of superoxide anion (O_2^-) and cytokines.[18-20] Another anti-inflammatory effect of statin drugs on monocytes and macrophages was the decrease of the expression of ICAM-1 and the secretion of IL-6 induced by lipopolysaccharides.[21] The presence of inflammation and endotoxin in the blood tends to upregulate leukocyte/endothelial interactions.[22] This is a stepwise process that requires that leukocytes first roll along the

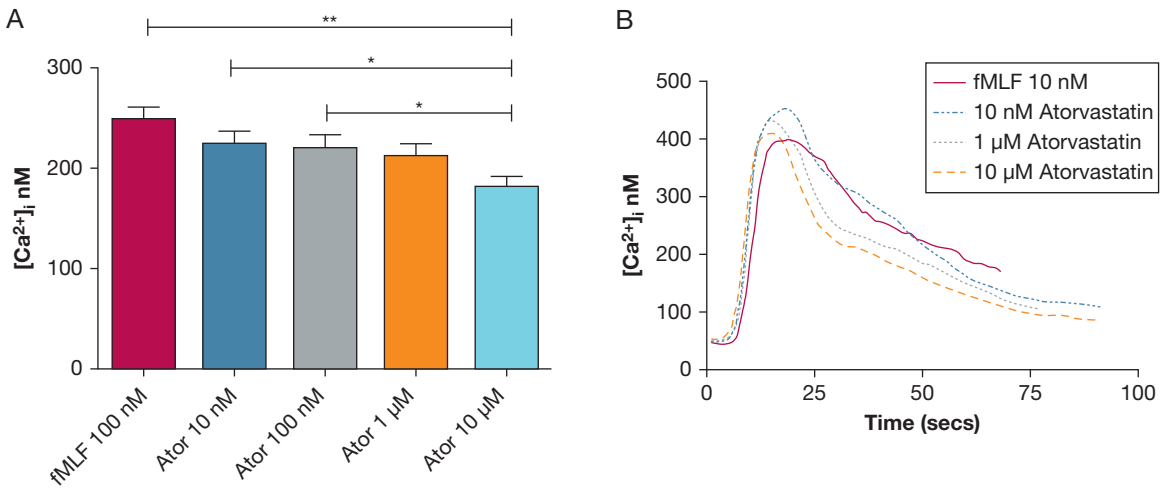

Figure 4 – *A, B, fMLF increases [Ca^{2+}] flux when added to serum neutrophils. This was reduced by atorvastatin in a concentration-dependent manner when neutrophils were pretreated with atorvastatin prior to adding fMLF. See Figure 3 legend for expansion of abbreviations.*

vascular endothelium mediated by CD62L followed by firm adhesion and migration into tissue mediated by CD11b. Firm adhesion involves the β_2 integrins CD11b and CD18, which are expressed on leukocytes and bind to the ICAM-1 on the vascular endothelium.[23]

As there was an atorvastatin-mediated reduction of ICAM-1 in our study, we explored the role of statin drugs on the expression of CD11b and CD62L. On activation with fMLF, there was an increase in CD11b expression and a decrease in CD62L expression, indicative of leukocyte activation and consequent selectin shedding. Atorvastatin significantly reduced fMLF-induced neutrophil activation and upregulation of CD11b. However, atorvastatin did not attenuate the shedding of CD62L. This might be explained if the inhibitory effect of statin drugs on activated neutrophils is mediated by blocking expression of the Mac-1 integrin subunit CD11b, rather than by inhibition of integrin activation. Similar findings were observed by Aparecida et al[24] in a study investigating the role of simvastatin on inflamed neutrophil adhesive properties.

Ca^{2+} ions serve as important second messengers in signal transduction, leading to the activation of downstream molecules.[25] Cytoplasmic levels of Ca^{2+} can increase either from release from internal calcium stores or by entry from outside the cell through calcium channels, leading to the rapid activation of molecules that promote function.[26] fMLF is recognized by neutrophils and is a potent neutrophil chemoattractant. fMLF, on binding to its heterotrimeric G-protein-coupled receptor, initiates signaling cascades that activate multiple pathways.[27] In neutrophils, stimulation by agonists that bind to the fMLF receptor trigger increases in intracellular Ca^{2+}. Elevation of intracellular free Ca^{2+} levels or mobilization of intracellular Ca^{2+} stores promotes neutrophil longevity.[28] The current study demonstrates that atorvastatin can reduce fMLF-mediated calcium flux. The implication of this in bronchiectasis could be that statin drugs, by decreasing calcium flux, reduce the longevity of neutrophils, with consequences for the persistence of the characteristic neutrophil inflammation.[29] Whether statin drugs can regulate extracellular Ca^{2+}mobilization by activation of store-operated calcium influx receptors or mobilize Ca^{2+} by release from intracellular stores through G-protein-coupled receptors or tyrosine kinase receptors remains to be determined.

There was no reduction in sputum inflammatory markers, and this was in keeping with our previous study.[5] Similar results were demonstrated by Llewellyn-Jones,[30] in which pretreatment with indomethacin led to a reduction in neutrophil chemotaxis but had no effect on sputum myeloperoxidase or free elastase activity. Further mechanistic studies are needed to assess the immunomodulatory effects of statin drugs on neutrophils.

One of the major side effects of statin drugs is myositis and liver function abnormalities. No patients had to withdraw because of myositis-induced leg pain or deranged alanine aminotransferase levels. Headaches

(6%) were the most important cause of dropouts, although this was noted only in the placebo group. There were no other adverse events recorded in the study, other than the ones mentioned, that necessitated patients to drop out.

Limitations

This study was not powered for the secondary end points. Another limitation was that the active and placebo drugs were not matched. However, the researcher was not aware of the study drugs administered to the patients, as these were dispensed directly by the pharmacy. The proof of concept study was short—3 months—and longer treatment of 6 months or more may be needed.

Conclusions

Using atorvastatin (80 mg daily for 3 months) to treat individuals with severe bronchiectasis who are chronically infected with *P aeruginosa* did not reduce cough severity when compared with placebo. However, treatment did improve reported quality of life and significantly reduced systemic inflammation. This study confirms the efficacy of atorvastatin as an anti-inflammatory agent in this clinical population and justifies larger multicenter studies.

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Additional information: The e-Appendix can be found in the Supplemental Materials section of the online article.

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